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THE METABOLISM OF TRIBUTYRIN

DISSERTATION

PRESENTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE GRADUATE SCHOOL OF THE OHIO STATE UNIVERSITY

BY

RUSSELL EDMUND DAVIS, B.A.

L.C.

The Ohio State University
1930

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PREFACE

The main object of the work reported in this paper was to study the chemical changes which tributyrin undergoes when administered to chickens by pipette, to rats as a part of a mixed diet up to about 9 per cent, and to rats subcutaneously and intraperitoneally. The digestibility of tributyrin by the hen was determined. The storage of tributyrin in the fat depots was determined by giving it to rats in a mixed diet and by injecting it subcutaneously and intraperitoneally. The influence of tributyrin on the acetone body excretion by diabetic rats was studied.

The writer wishes to thank Doctor J. F. Lyman of The Ohio State University for helpful suggestions and criticisms.

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THE METABOLISM OF TRIBUTYRIN.

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(Received for publication, April 28, 1930.)

The digestibility of the glycerides of the lower fatty acids, their deposition in the fat depots, and their final transformations in the body are matters of considerable uncertainty. Butyric acid, if present in natural fats, exists not as tributyrin but rather as mixed glycerides. If tributyrin could be fed in quantity, its effect on depot fat and on the excretion of acetone bodies would give information of value. But tributyrin is intensely bitter and decidedly toxic. Animals will not willingly eat large amounts of it. Eckstein (1) found that butyric acid could be fed to rats in the form of sodium butyrate up to 19 per cent of the ration. He found the butyryl radical was not deposited in the fat tissues. Our work confirms this.

We have been able to administer tributyrin in three ways: (1) to chickens by pipette, (2) to rats as a part of a mixed ration up to about 9 per cent, and (3) to rats subcutaneously or intraperitoneally.

Digestion.

The digestibility of tributyrin was determined by feeding it to chickens. Rats could not be used because they scattered so much feed that it was impossible to separate it quantitatively from the feces. Any amount of tributyrin could be given chickens by pipette. By using this technique there was no chance of getting any tributyrin in the feces except the undigested portion which was excreted.

The procedure used is as follows: The chickens were kept in metabolism cages which were set over large evaporating dishes. The feces were collected at regular intervals and the feed collected and weighed.

The basal ration was kept before the chickens at all times. This basal ration contained per 75 gm.:

	gm.
Meat scrap (ether-extracted)	18.0
Starch	42.5
Cod liver oil	0.5
Yeast	10.0
Osborne and Mendel salt mixture (2)	4.0

The amounts of tributyrin given varied with the amount of feed eaten and with the time of the experiment. In general the tributyrin made up about 18 per cent of the feed consumed.

The feces were dried, ground, and extracted with ether. The ether extract was saponified with alcoholic potassium hydroxide. The alcohol was evaporated off and the solution acidified with sulfuric acid, and steam-distilled. The volatile, water-soluble fatty acids were collected, titrated, and calculated as butyric acid. Blank determinations were made on the basal ration. The results given in Table I have been corrected by subtracting this blank. Tributyrin was added to feces and subjected to the same procedure, and it was possible to recover from 97 to 99 per cent of the butyric acid.

Tributyrin was toxic to chickens. Lesions were found in the proventriculus and in the gizzard. The intestinal walls were inflamed and in some cases the mucosa apparently had been destroyed in the fore part of the small intestine. There was some hemorrhage, blood being detected in the feces. In every case the gallbladder was greatly enlarged and often bile had been regurgitated into the gizzard.

Table I gives the results of a set of digestibility determinations. It is seen that tributyrin is digested by chickens. The digestibility falls off if the experiment is repeated with the same chicken. This is probably due to the toxic effect of tributyrin and to damage to the digestive organs.

Storage of Tributyrin.

Rats weighing about 100 gm. were used in these experiments. They were caged individually and weighed every 3 or 4 days. The food was weighed at the same time. The experiments ran about

3 weeks. The diets used in these experiments are given in Table II, and food consumption and body weights in Table III.

The rats were killed by an overdose of chloroform and the depot fat was removed by dissection. This fat together with the hide

TABLE I.

Digestibility of Tributyrin in the Hen.

Hen No.	Basal ration fed.	Tributyrin fed.	Tributyrin fed, per cent of ration.	Tributyrin recovered in feces.	Tributyrin digested.	Time of experiment.
	gm.	*gm.*		*gm.*	*per cent*	*hrs.*
1a	41.3	9.6	18.8	0.71	92.5	48
1b	58.5	10.2	15.0	2.7	73.5	48
2	71.2	19.7	21.6	1.0	95.0	72
3	46.6	31.7*	40.5	3.9	87.8	48
4	55.0	9.9	15.2	1.5	84.9	48
5a	43.5	10.1	19.0	0.2	98.0	48
5b	47.8	11.2	19.0	2.6	76.8	48

* 10 cc. of tributyrin were given every 6 hours.

TABLE II.

Composition of Diets.

The quantities of the substances are expressed in gm.

Feed.	Diet No.					
	1	2	3	4	5	6
Tributyrin	9	9		9		9
Lard	52	49	61		68	58
Starch	10	10	10	62		
Casein	20	20	20	20	20	20
Yeast	5	5	5	5	8	8
Salts*	4	4	4	4	4	4
Cod liver oil		3				
Ground paper	2	2	2	2	2	2

* Osborne and Mendel salt mixture (2).

was extracted with an alcohol-ether mixture which consisted of 3 parts of alcohol to 1 part of ether (3). The alcohol and ether were evaporated off at reduced pressure and the residue extracted with anhydrous ether. This fat was used in the saponification number determinations which were made according to the official method (4). The results of these experiments are given in Table IV.

Because of the toxic effect of the tributyrin it was impossible to feed large quantities of it. In order to give rats large quantities

TABLE III.

Food Consumption and Body Weights of Rats Fed with and without Tributyrin.

Rat No.	Initial weight.	Final weight.	Feeding period.	Food consumed.	Diet No.
	gm.	*gm.*	*days*	*gm.*	
1	148	161	21	138	1
2	116	112	21	111	1
3	127	149	21	137	1
4	121	122	21	108	1
17	192	206	25	172	1
19	130	158	25	138	1
7	108	127	21	128	2
8	131	142	21	131	2
9	94	109	21	117	2
11	161	170	25	162	3
12	186	219	25	216	3
13	173	195	25	200	3
14	188	195	25	207	4
15	185	180	25	212	4
16	179	195	25	188	4

TABLE IV.

Effect of Tributyrin Feeding on the Composition of Depot Fat in the White Rat.

Diet No.	Rat No.	Weight of sample.	KOH (0.5 N) required for saponification.	Saponification No.
		gm.	*cc.*	
1	1, 2, 3, 4	2.542	17.6	193.5
		2.243	15.4	193.0
1	17, 19	2.172	15.4	198.5
		1.844	12.8	197.0
2	7, 8, 9	2.695	18.3	190.5
		1.522	10.3	191.0
3	11, 12, 13	2.891	20.2	196.0
		2.220	15.2	194.0
4	14, 15, 16	2.426	17.1	197.5
		2.620	18.2	195.0

of tributyrin it was injected subcutaneously and intraperitoneally. 2 cc. of tributyrin were injected daily for varying lengths of time.

The rats were killed by an overdose of chloroform and before the fat was removed all unabsorbed tributyrin was carefully washed off. The following tabulation gives the saponification numbers which were obtained in these experiments.

Rat No.	Tributyrin injected.	Saponification No.	Injection.
	cc.		
31	4	288.8	Subcutaneous.
32	4	283.8	"
33	4	280.5	Intraperitoneal.
34	4	285.6	"

10 cc. of tributyrin were injected intraperitoneally into a large vigorous rat. 2 hours later the rat was found dead. On autopsy much of the tributyrin was recovered from the body cavity. The internal organs appeared to be normal. The experiment was repeated and the rat observed continuously after the injection of the tributyrin.

The tributyrin had a decided anesthetic action. In about 15 minutes anesthesia was complete. The rate of breathing became very slow and ceased entirely 1 hour and 12 minutes after the injection of the tributyrin. On autopsy the heart was found to be tightly contracted. The internal organs appeared to be normal. The experiment was repeated with similar results, the rat dying in less than $1\frac{1}{2}$ hours.

The fat of these animals was examined as in previous experiments and the saponification number was the same as that of normal rat fat. Because of the short time between the injection of the tributyrin and the death of the animal one would not expect much fat storage.

Effect of Tributyrin on Formation of Acetone Bodies.

According to the β-oxidation theory a diabetic animal should excrete more acetone bodies when fed tributyrin than when fed an equal amount of fats having longer fatty acid chains. We tested this by using rats which were rendered diabetic by phlorhizin. Diets 1, 3, 5, and 6, whose composition is given in Table II, were used in these experiments.

The procedure of Levine and Smith (5) was followed with a

few modifications. The rats were kept in cages so constructed that the urine could be collected under mineral oil. A solution of sodium fluoride was used as a preservative for the urine. The urine was collected every 2nd day and was immediately analyzed. The phlorhizin was suspended in olive oil and was injected daily. The daily dose was 25 mg. for the first series and 37.5 mg. for the second series of experiments.

The acetone bodies were determined according to the method of Van Slyke (6). β-Hydroxybutyric acid, acetoacetic acid, and acetone were determined together and reported as acetone. Sugar and nitrogen were determined in several samples. Sugar was determined by the Bertrand-Walker-Munson (7) method. A modified Kjeldahl method was used to determine the total nitrogen.

DISCUSSION.

Tributyrin is digested by the hen but the digestibility falls off if the same hen is used for a second trial. The average digestibility for all determinations was 86.9 per cent. The average of five first trials was 91.6 per cent. It is believed that this decrease in digestibility is due to injury of the digestive tract by the tributyrin.

It is interesting to note that during the steam distillation of hen feces for the determination of butyric acid insoluble volatile fatty acids were obtained along with the soluble volatile acids. The amount of insoluble volatile acids was less in the blank determinations than in the experiments in which tributyrin was fed. This suggests that some synthesis of higher fatty acids had taken place in the intestine.

When tributyrin was injected into rats there was an increase in the saponification number of the depot fat, which indicates that there is some storage of tributyrin. There was not a great deal of difference between results with subcutaneous and intraperitoneal injections; the saponification number was raised to over 280.0 in every case. On the other hand when tributyrin was fed there was no increase in the saponification number of the depot fat. These results indicate that there is no storage of tributyrin when it is fed.

Since tributyrin was digested and absorbed and was not stored in the animal body, what was its fate? There are several possible answers to this question. The butyric acid might have been trans-

formed to higher fatty acids as it passed through the intestinal wall or elsewhere, it might have been oxidized at once and spared other fats to be stored, or thirdly, it might have been converted to glucose. Our experiments lead us to believe that the butyric acid was transformed in part to higher fatty acids. From the fact that the injection of tributyrin increased the saponification number of the depot fat, while the feeding of it did not, it seems probable that the fat is transformed before entering the blood. The presence of increased amounts of insoluble volatile fatty acids in the feces after feeding the tributyrin might be explained on the basis that higher fatty acids are being built up in this transformation. Leube (8) found that the Reichert-Meissl number of the depot fat of a dog did not change when large quantities of butter were fed. Hughes (9) observed that short chain fatty acids were not stored in the animal body. All of this evidence supports the view that the fat is transformed but of course does not describe the mechanism of the change. An increased excretion of acetone bodies following tributyrin feeding with starch-free diets indicates that a part of the absorbed tributyrin was rapidly oxidized. The amount of glucose excreted by phlorhizinized rats fed tributyrin does not indicate a conversion of butyrin to glucose. It was observed that the presence of carbohydrates in a diet high in lard has a marked antiketogenic effect in the phlorhizinized as well as in the normal rat. For example, the removal of the starch from the diet raised the acetone body excretion from 0.57 mg. per 2 day period to 1.6 mg. in the normal, and from 28.8 to 161.0 mg. in the phlorhizinized animal. A probable explanation for the antiketogenic effect of carbohydrate in the phlorhizinized rat is that the phlorhizinized animal is still able to oxidize carbohydrates and when carbohydrates are being oxidized the normal oxidation of fats can occur also. The substitution of tributyrin for lard to the extent of 9 per cent had no marked effect on acetone body excretion provided 10 per cent of starch also was in the food mixture. A rat receiving no tributyrin excreted 0.57 and 28.8 mg. of acetone bodies per 2 day period without and with phlorhizin respectively. A rat that received tributyrin similarly excreted 0.50 and 26.6 mg. When the diet was very low in carbohydrate, containing only small amounts from the yeast and impure casein, but was high in lard, the average excretion of acetone bodies per 2 day

period was 2.1 mg. when no phlorhizin was given and 196.9 mg. when phlorhizin was administered. When tributyrin was added to the starch-free diet the acetone body excretion per 2 day period averaged 2.9 and 324.8 mg. without and with phlorhizin respectively.

SUMMARY.

The average digestibility of tributyrin as determined by seven trials with five hens was 86.9 per cent. If two second trials are excluded the average of the first trials was 91.9 per cent. The toxicity of tributyrin is believed to be the cause of the decrease in digestibility when the same hen is used for the second trial.

There was some storage of tributyrin in the depot fat when it was injected subcutaneously or intraperitoneally. The saponification number of the depot fat from injected rats was about 285, that from control rats was 195.

No tributyrin was stored in the depot fat after tributyrin was fed. It is believed that the fat was transformed in part as it passed through the intestinal wall. The remainder probably was oxidized rapidly.

The amount of acetone bodies excreted by both normal and phlorhizinized rats increased when carbohydrate was removed from the diet. When tributyrin was fed the excretion of acetone bodies increased, provided the diet used was very low in carbohydrate; otherwise tributyrin had no such effect. These facts are in agreement with the theory of β-oxidation of the fatty acids and the antiketogenic effect of carbohydrate metabolites.

The quantitative relations between carbohydrate oxidation and fat oxidation that fix a maximum ketogenic-antiketogenic ratio for man beyond which acetone bodies appear in the urine in large amounts do not hold at all for the rat.

It is a pleasure to acknowledge indebtedness to Professor J. F. Lyman for valuable advice and criticism.

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AUTOBIOGRAPHY

I, Russell Edmund Davis, was born in Leesburg, Ohio, January 7, 1903. My secondary education was obtained in the public schools of Leesburg, Ohio; my undergraduate education in Wilmington College and The Ohio State University where I received the degree of Bachelor of Arts in 1925. From 1925 to 1928 inclusive I gave full time to graduate study at The Ohio State University, my major being Agricultural Chemistry. During the year 1927–28 I was University Fellow in Agricultural Chemistry. I received the degree of Doctor of Philosophy at the end of the spring quarter of 1928.

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